

CLAIMS

1. A polynucleotide encoding a cytochrome P-450 enzyme of an alkaloid producing poppy plant involved in a step in an alkaloid biosynthesis pathway of the plant and which inhibits accumulation of at least one benzyloquinoline alkaloid above a normal level of the alkaloid in the plant, or which encodes an active fragment, homologue or variant thereof having enzymatic activity of the enzyme.
2. A polynucleotide according to claim 1 wherein in the alkaloid biosynthesis pathway is a biosynthesis pathway for the synthesis of a phenanthrene alkaloid and the enzyme inhibits the benzyloquinoline alkaloid from accumulating with the phenanthrene alkaloid in the poppy plant.
3. A polynucleotide according to claim 2 wherein the phenanthrene alkaloid is selected from the group consisting of morphine, codeine, codeinone, thebaine and oripavine.
4. A purified polynucleotide according to any one of claims 1 to 3 selected from the group consisting of:
 - (a) a polynucleotide comprising nucleotides 222 to 2318 of figure 1 (SEQ ID No:1) or a partial nucleotide sequence thereof;
 - (b) a polynucleotide which is degenerate to a polynucleotide of (a); and
 - (c) a polynucleotide having at least 60% sequence identity with a polynucleotide of (a) or (b).
5. A polynucleotide according to claim 4 wherein the polynucleotide of (c) has at least 75% sequence identity with a polynucleotide of (a) or (b).
6. A polynucleotide according to claim 4 or 5 further comprising one or more nucleotide sequences selected from the group consisting of a regulatory control sequence, a leader sequence for directing location of the enzyme in an intracellular environment or transport of the enzyme to an extracellular environment, and untranslated sequences.
7. A polynucleotide according to claim 6 wherein the untranslated sequence is selected from the group consisting of introns, and 5' and 3' untranslated sequences.

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8. A polynucleotide according to any one of claims 1 to 7 wherein the enzymatic activity of the enzyme inhibits accumulation of the benzyloquinoline alkaloid above 25% of the normal level of the alkaloid in the poppy plant.
- 5 9. A polynucleotide according to any one of claims 1 to 8 wherein the at least one benzyloquinoline alkaloid is selected from the group consisting of (S)-reticuline, laudanine, laudanosine and codamine.
10. A polynucleotide according to any one of claims 1 to 9 wherein the alkaloid producing poppy plant is a member of the plant sub-family *Papaveroideae*.
- 10 11. A polynucleotide according to claim 10 wherein the alkaloid producing poppy plant is *P. somniferum*.
12. A purified polynucleotide which is complementary to all or a region of a polynucleotide encoding a cytochrome P-450 reductase enzyme of an alkaloid producing poppy plant involved in a step in an alkaloid biosynthesis pathway of the plant and the enzymatic activity of which inhibits accumulation of at least one
15 benzyloquinoline alkaloid above a normal level of the alkaloid in the plant, or which is capable of hybridising with the polynucleotide encoding the enzyme under cellular conditions such that expression of the enzyme is down-regulated.
13. A polynucleotide according to claim 12 wherein the alkaloid biosynthesis pathway is a biosynthesis pathway for the synthesis of a phenanthrene alkaloid and the enzyme
20 inhibits the benzyloquinoline alkaloid from accumulating with the phenanthrene alkaloid in the poppy plant.
14. A polynucleotide according to claim 13 wherein the phenanthrene alkaloid is selected from the group consisting of morphine, codeine, codeinone, thebaine and oripavine.
15. A polynucleotide according to any one of claims 12 to 14 wherein the polynucleotide is complementary to all or a region of a polynucleotide selected from the group
25 consisting of:
- (a) a polynucleotide comprising nucleotides 222 to 2318 of figure 1 (SEQ ID No:1) or a partial nucleotide sequence thereof;
- (b) a polynucleotide which is degenerate to a polynucleotide of (a); and
30 (c) a polynucleotide having at least 60% sequence identity with a polynucleotide of (a) or (b).

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16. A polynucleotide according to claim 15 wherein the polynucleotide of (c) has at least 75% sequence identity with the polynucleotide of (a) or (b).
17. A polynucleotide according to claim 15 or 16 further comprising one or more nucleotide sequences selected from the group consisting of a regulatory control sequence, a leader sequence for directing location of the enzyme in an intracellular environment or transport of the enzyme to an extracellular environment, and untranslated sequences.
18. A polynucleotide according to claim 17 wherein the untranslated sequence is selected from the group consisting of introns, and 5' and 3' untranslated sequences.
19. A polynucleotide according to any one of claims 12 to 18 wherein the enzymatic activity of the enzyme inhibits accumulation of the benzyloquinoline alkaloid above 25% of the normal level of the alkaloid in the poppy plant.
20. A polynucleotide according to any one of claims 12 to 19 wherein the at least one benzyloquinoline alkaloid is selected from the group consisting of (S)-reticuline, laudanine, laudanosine and codamine.
21. A polynucleotide according to any one of claims 12 to 20 wherein the alkaloid producing poppy plant is a member of the plant sub-family *Papaveroideae*.
22. A polynucleotide according to claim 21 wherein the alkaloid producing poppy plant is *P. somniferum*.
23. A recombinant vector incorporating a polynucleotide as defined in any one of claims 1 to 11.
24. A vector according to claim 23 wherein the vector is an expression vector for transcription of the polynucleotide.
25. A recombinant vector incorporating a polynucleotide as defined in any one of claims 12 to 22.
26. A vector according to claim 25 wherein the vector is an expression vector for transcription of the polynucleotide.

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27. A purified cytochrome P-450 reductase enzyme encoded by a polynucleotide as defined in any one of claims 1 to 11, or an active fragment, homologue, analogue, derivative, or variant thereof having enzymatic activity of the enzyme.
- 5 28. A host cell incorporating a polynucleotide as defined in any one of claims 1 to 11 or a recombinant vector as defined in claim 23 or 24.
29. A cell according to claim 28 wherein the cell is a plant cell.
30. A cell according to claim 29 wherein the plant cell is a cell of a plant of the plant sub-family *Papaveroideae*.
31. A cell according to claim 30 wherein the plant cell is a plant cell from *P. somniferum*.
- 10 32. A host cell incorporating a polynucleotide as defined in any one of claims 12 to 22 or a recombinant vector as defined in claim 25 or 26.
33. A cell according to claim 32 wherein the cell is a plant cell.
34. A cell according to claim 33 wherein the plant cell is a cell of a plant of the plant sub-family *Papaveroideae*.
- 15 35. A cell according to claim 34 wherein the plant cell is a plant cell from *P. somniferum*.
36. A method for providing an alkaloid producing poppy plant with an altered alkaloid content, the method comprising up-regulating or down-regulating activity of a cytochrome P-450 reductase enzyme of the plant that inhibits accumulation of at least one benzyloquinoline alkaloid above a normal level of the alkaloid in the plant.
- 20 37. A method according to claim 36 wherein the enzyme is involved in a step in an alkaloid biosynthesis pathway of the poppy plant for the synthesis of a phenanthrene alkaloid and inhibits the benzyloquinoline alkaloid from accumulating with the phenanthrene alkaloid in the poppy plant.
38. A method according to claim 37 wherein the phenanthrene alkaloid is selected from the group consisting of morphine, codeine, codeinone, thebaine and oripavine.
- 25 39. A method according to any one of claims 36 to 38 comprising transforming the plant with a polynucleotide encoding the enzyme, or an active fragment, homologue, or

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variant thereof having the activity of the enzyme and wherein the polynucleotide is expressed.

40. A method according to claim 39 wherein the polynucleotide is selected from the group consisting of:

- 5 (a) a polynucleotide comprising nucleotides 222 to 2318 of figure 1 (SEQ ID No:1) or a partial nucleotide sequence thereof;
- (b) a polynucleotide which is degenerate to a polynucleotide of (a); and
- (c) a polynucleotide having at least 60% sequence identity with a polynucleotide of (a) or (b).

10 41. A method according to claim 40 wherein the polynucleotide of (c) has at least 75% sequence identity with a polynucleotide of (a) or (b).

42. A method according to claim 40 or 41 wherein the polynucleotide comprises one or more untranslated sequences.

15 43. A method according to any one of claims 36 to 38 wherein the down-regulation of the activity of the enzyme comprises transforming the plant with a polynucleotide which effects down-regulation of expression of the enzyme.

44. A method according to claim 43 wherein the down-regulation of expression of the enzyme comprises substantially silencing the expression of the enzyme.

20 45. A method according to claim 43 or 44 wherein the polynucleotide comprises double stranded nucleic acid, or is transcribed to yield double stranded nucleic acid, which effects the down-regulation of expression of the enzyme.

46. A method according to claim 45 wherein the double stranded nucleic acid comprises hairpin RNA.

25 47. A method according to any one of claims 36 to 46 wherein the at least one benzylisoquinoline alkaloid is selected from the group consisting of (S)-reticuline, laudanine, laudanosine and codamine.

48. A method according to any one of claims 36 to 47 wherein the alkaloid producing poppy plant is a member of the plant sub-family *Papaveroideae*.

30 49. A method according to claim 48 wherein the alkaloid producing poppy plant is *P. somniferum*.

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50. A method for providing a transgenic plant, the method comprising:

transforming a plant cell with a polynucleotide for expression of a cytochrome P-450 reductase enzyme of an alkaloid producing poppy plant involved in a step in an alkaloid biosynthesis pathway of the poppy plant and which inhibits accumulation of at least one benzylisoquinoline alkaloid above a normal level of the alkaloid in the poppy plant, or an active fragment, homologue, or variant thereof having enzymatic activity of the enzyme, or with a polynucleotide for down-regulating expression of the enzyme;

culturing the transformed plant cell to produce cultured cells; and

generating the transgenic plant from the cultured cells wherein cells of the transgenic plant contain the polynucleotide.

51. A method according to claim 50 wherein the plant cell is a plant cell of a plant which expresses the enzyme.

52. A method according to claim 50 or 51 wherein the polynucleotide is selected from the group consisting of:

(a) a polynucleotide comprising nucleotides 222 to 2318 of figure 1 (SEQ ID No:1) or a partial nucleotide sequence thereof;

(b) a polynucleotide which is degenerate to a polynucleotide of (a); and

(c) a polynucleotide having at least 60% sequence identity with a polynucleotide of (a) or (b).

53. A polynucleotide sequence according to claim 52 wherein the polynucleotide of (c) has at least 75% sequence identity with a polynucleotide of (a) or (b).

54. A method according to claim 52 or 53 wherein the polynucleotide comprises one or more untranslated sequences.

55. A method according to claim 50 or 51 wherein the polynucleotide comprises a polynucleotide complementary to all or a region of a nucleic acid sequence of the plant cell which encodes the enzyme.

56. A method according to claim 55 wherein the polynucleotide is complementary to all or a region of a polynucleotide selected from the group consisting of:

(a) a polynucleotide comprising nucleotides 222 to 2318 of figure 1 (SEQ ID No:1) or a partial nucleotide sequence thereof;

(b) a polynucleotide which is degenerate to a polynucleotide of (a); and

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(c) a polynucleotide having at least 60% sequence identity with a polynucleotide of (a) or (b).

57. A method according to claim 56 wherein the polynucleotide comprises one or more untranslated sequences.

5 58. A method according to any one of claims 55 to 57 wherein the polynucleotide substantially silences the expression of the enzyme.

59. A method according to claim 50 or 51 wherein the polynucleotide comprises a sense sequence linked to an antisense sequence sufficiently complementary to the sense sequence for hybridisation and thereby formation of double stranded nucleic acid for
10 effecting the down-regulation of expression of the enzyme.

60. A method according to any one of claims 50 to 59 wherein the plant cell is a cell of a plant which is a member of the plant sub-family *Papaveroideae*.

61. A method according to claim 60 wherein the plant is *P. somniferum*.

15 62. A transgenic plant having cells transformed or transfected with a polynucleotide for expression of a cytochrome P-450 reductase enzyme of an alkaloid producing poppy plant involved in a step in an alkaloid biosynthesis pathway of the poppy plant and which inhibits accumulation of at least one benzyloquinoline alkaloid above a normal level of the alkaloid in the poppy plant, or an active fragment, homologue, analogue or variant thereof having enzymatic activity of the enzyme, or with a
20 polynucleotide for down-regulating expression of the enzyme, or seed, reproductive material, progeny or a descendant of the transgenic plant incorporating the polynucleotide.

63. A transgenic plant according to claim 62 wherein the polynucleotide is selected from the group consisting of:

- 25 (a) a polynucleotide comprising nucleotides 222 to 2318 of figure 1 (SEQ ID No:1) or a partial nucleotide sequence thereof;
- (b) a polynucleotide which is degenerate to a polynucleotide of (a); and
- (c) a polynucleotide having at least 60% sequence identity with a polynucleotide of (a) or (b).

30 64. A transgenic plant according to claim 63 wherein the polynucleotide of (c) has at least 75% sequence identity with the polynucleotide of (a) or (b).

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65. A transgenic plant according to claim 63 or 64 wherein the polynucleotide comprises one or more untranslated sequences.
66. A transgenic plant according to any one of claims 62 to 64 wherein the polynucleotide is expressed and the transgenic plant thereby exhibits an altered alkaloid content.
- 5 67. A transgenic plant according to claim 66 wherein the altered alkaloid content comprises increased accumulation of at least one phenanthrene alkaloid.
68. A transgenic plant according to claim 67 wherein the phenanthrene alkaloid is selected from the group consisting of morphine, oripavine, thebaine, codeine and codeinone.
- 10 69. A transgenic plant according to claim 62 wherein the polynucleotide is complementary to all or a region of a polynucleotide selected from the group consisting of:
- (a) a polynucleotide comprising nucleotides 222 to 2318 of figure 1 (SEQ ID No:1) or a partial nucleotide sequence thereof;
- 15 (b) a polynucleotide which is degenerate to a polynucleotide of (a); and
- (c) a polynucleotide having at least 60% sequence identity with a polynucleotide of (a) or (b).
70. A transgenic plant according to claim 69 wherein the polynucleotide of (c) has at least 75% sequence identity with the polynucleotide of (a) or (b).
- 20 71. A transgenic plant according to claim 69 or 70 wherein the polynucleotide selected from (a) to (c) comprises one or more untranslated sequences.
72. A transgenic plant according to claim 62 wherein the polynucleotide is transcribed to generate double stranded nucleic acid which effects the down-regulation of expression of the enzyme.
- 25 73. A transgenic plant according to any one of claims 69 to 72 wherein the polynucleotide substantially silences the expression of the enzyme.
74. A transgenic plant according to any one of claims 69 to 73 wherein the transgenic plant exhibits altered accumulation of at least one alkaloid selected from (S)-reticuline, laudanine, laudanosine and codamine.

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75. A transgenic plant according to any one of claims 62 to 74 wherein the at least one benzyloisoquinoline alkaloid is selected from the group consisting of (S)-reticuline, laudanine, laudanosine and codamine.
- 5 76. A transgenic plant according to any one of claims 62 to 75 wherein the transgenic plant is a member of the plant sub-family *Papaveroideae*.
77. A transgenic plant according to claim 76 where the transgenic plant is *P. somniferum*.
78. An alkaloid producing poppy plant with an altered alkaloid content provided by a method as defined in any one of claims 36 to 49.
79. A transgenic plant provided by a method as defined in any one of claims 50 to 61.
- 10 80. Straw extracted from a transgenic plant as defined in any one of claims 62 to 77.
81. Latex or opium extracted from a transgenic plant as defined in any one of claims 62 to 77.
82. An alkaloid extracted from straw as defined in claim 80 or latex or opium as defined in claim 81.
- 15 83. An alkaloid according to claim 82 wherein the alkaloid is selected from the group consisting of phenanthrene alkaloids and benzyloisoquinoline alkaloids.
84. An alkaloid according to claim 82 wherein the alkaloid is selected from the group consisting of morphine, oripavine, thebaine, codeinone, codeine, (S)-reticuline, laudanosine, laudanine and codamine.
- 20 85. An alkaloid concentrate extracted from straw as defined in claim 80 or latex or opium as defined in claim 81.
86. An alkaloid concentrate according to claim 85 comprising a plurality of alkaloids with at least one of the alkaloids being selected from the group consisting of phenanthrene alkaloids and benzyloisoquinoline alkaloids.
- 25 87. A method for providing at least one poppy plant alkaloid or alkaloid concentrate, the method comprising:
harvesting capsules of a transgenic alkaloid producing poppy plant to obtain straw or latex; and

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extracting the alkaloid or alkaloid concentrate from the straw or latex;

wherein the alkaloid producing poppy plant has cells transformed with a polynucleotide that up-regulates expression of a cytochrome P-450 reductase enzyme involved in a step in an alkaloid biosynthesis pathway of the poppy plant and the enzymatic activity of which inhibits accumulation of at least one benzyloquinoline alkaloid above a normal level of the alkaloid in the poppy plant, or an active fragment, homologue, or variant thereof having activity of the enzyme, or with a polynucleotide which down-regulates expression of the enzyme in the poppy plant, such that the straw or latex thereby has an altered alkaloid content.

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- 10 88. A method according to claim 87 wherein the polynucleotide effects down-regulation of the expression of the enzyme and the straw or latex thereby has an increased content of the benzyloquinoline alkaloid relative to one or more other alkaloids in the straw or latex.
- 15 89. A method according to claim 88 wherein the alkaloid extracted from the straw or latex is the benzyloquinoline alkaloid.
90. A method according to claim 88 wherein the alkaloid concentrate reflects the increased content of the benzyloquinoline alkaloid relative to the one or more other alkaloids.
- 20 91. A method according to any one of claims 87 to 90 wherein the benzyloquinoline alkaloid is selected from a group consisting of (S)-reticuline, laudanosine, laudanine and codamine.
- 25 92. A method according to claim 87 wherein the enzyme inhibits accumulation of the benzyloquinoline alkaloid with at least one phenanthrene alkaloid in the plant and the polynucleotide effects up-regulation of enzymatic activity of the enzyme such that the straw or latex has an increased content of the phenanthrene alkaloid relative to one or more other alkaloids in the straw or latex.
93. A method according to claim 92 wherein the alkaloid extracted from the straw or latex is the phenanthrene alkaloid.
- 30 94. A method according to claim 92 wherein the alkaloid concentrate reflects the increased content of the phenanthrene alkaloid relative to the one or more other alkaloids.

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- 95. An alkaloid provided by a method as defined in claim 87.
- 96. An alkaloid concentrate provided by a method as defined in claim 87.
- 97. The microorganism deposited with the National Measurement Institute under Accession No. NM04/43447.